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THE NUMBER OF CELLULAR ELEMENTS IN MILK.*

ROBERT S. BREED AND I. READ STIDGER.

(From the Biological Laboratory, Allegheny College, Meadville, Pa.)

The new method for determining the number of cellular elements in milk, recently devised by Prescott and Breed (1910), reveals such an inaccuracy in the older methods for determining the number of these cells that it again raises the question of how many body cells may be expected in milk under normal and pathological conditions. On this account, an investigation was undertaken to determine the number of cells present under a variety of conditions as a preliminary to experimental work to determine the significance of these cells in milk and the cause of the variations which are known to occur.

HISTORICAL SURVEY.

No attempt will be made here to criticize the older methods for determining the number of cells present in milk, as that will be done in another paper.[1] The question of the origin of these cells is in dispute at the present time, but the drift of opinion seems to be toward ascribing an epithelial, rather than a leukocytic, origin to them. On account of this dispute only noncommittal terms will be used in this paper.

A considerable amount of work has been done by the so-called "smeared-sediment" (Stokes-Stewart-Slack) method and various "volumetric" methods to determine the number of cells present in milk under a variety of conditions. However, it is generally admitted that the smeared-sediment method even in its best form does not yield sufficiently accurate results to give more than a general impression of the number of cells present, if indeed this be true. On this account, very little reference will be made in this review to work done by this method. Just how accurate the results of the various volumetric methods may be remains to be seen, but in any case they are regarded as being of sufficient importance to summarize them at this place. The earliest of these methods is the Doane-Buckley, which has recently been modified by Russell and Hoffman. This modification is the method recommended by the Committee on Standard Methods of Bacterial Milk Analysis appointed by the Ameri-

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[1] "The Effect of the Centrifuge and Separator on the Distribution of Cellular Elements in Milk with a Criticism of the Present Methods for Determining the Number of Such Cells in Milk" (R. S. Breed). This paper will probably be published in the *Milchwirtsch. Centralbl.* for 1911.

can Public Health Association.[1] An account of all of the methods mentioned above is given in the committee's publication. Other volumetric methods, based on a similar principle, have been devised by Savage (1906) and Hewlett, Villar, and Revis (1910). The test devised by Trommsdorff has been the most popular in European countries but the results of this test are not expressed in numbers of cells per cubic centimeter, and the method itself is open to all of the criticism that has been directed against the smeared-sediment method, so that the work done by this method is not referred to here.

a. The most important point in connection with these cells is the determination of the number of cells present and the variation in number to be expected in the milk of individual normal cows. A large amount of work has already been done by various men to determine these points. Unfortunately, the authors quoted do not always state clearly whether or not the sample examined was the mixed milk of the entire milking of the individual cow. The statements of the various authors regarding the question whether or not the cows examined were normal are unsatisfactory. No one, not even a competent veterinarian, can say what an entirely normal udder is, and the finding of an udder that is more fibrous or fleshy than usual is not proof in itself that the secretion from it will be abnormal. All figures given in this review represent the number of cells per cubic centimeter.

Doane (1905) (p. 210, Doane-Buckley test) gives the record of 25 cows from the Maryland Agricultural Experiment Station herd as follows:

December 15, 1904, 23 out of 25 cows: average 123,000, maximum 1,000,000, minimum 4,000.

January 4, 1905, 22 out of 25 cows: average 168,000, maximum 1,600,000, minimum 3,000.

March 1, 1905, 16 out of 25 cows: average 91,000, maximum 300,000, minimum 8,000.

On p. 211, he gives the record for 102 cows from "one of the best and most carefully kept herds in the United States": average 252,000, maximum 4,600,000, minimum 2,000.

Savage (1906) (p. 130, Savage test) gives the record for 40 cows where only the middle milk of the milking was used in obtaining the sample. "As a rule no selection was exercised in picking out the cow to be examined. In several instances, however, cows recently calved and just readmitted to the herd for milking purposes were chosen; in one or two instances the cowkeeper was asked to select his finest and healthiest cows." All of the cows were kept in Colchester Borough, England: 40 cows averaged 826,575, maximum 4,380,000, minimum 35,000.

Ward, Henderson, and Haring (1906) (p. 150, Doane-Buckley test) give a set of figures for 87 healthy cows from California: average 49,000, maximum 581,000, minimum 2,000. On pp. 152–54 they give figures for 71 cows, selected because of udder troubles or for other reasons which might be expected to give high counts: average 260,000, maximum 4,800,000, minimum 3,000. The tests from five other cows were not counted because the cells were badly clumped or innumerable.

Russell and Hoffman (1907*a*) (Doane-Buckley test and their modification of this test) have made the most thorough and satisfactory investigations along this line, giving, as they do, a series of examinations of animals whose clinical history was known, some being normal and others not. The results of numerous tests of 18 normal animals (p. 244) show 31.1 per cent under 50,000, 18.5 per cent between 50,000 and

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[1] *Am. Jour. Pub. Hyg.*, 1910, 20, p. 315.

100,000, 36.6 per cent between 100,000 and 500,000, 10.3 per cent between 500,000 and 1,000,000, and 3.5 per cent above 1,000,000: maximum 2,191,250, minimum 1,250. A similar series of tests for 11 animals showing more or less pathological conditions but giving apparently normal milk give 12.9 per cent under 50,000, 15.2 per cent between 50,000 and 100,000, 47.6 per cent between 100,000 and 500,000, 15 per cent between 500,000 and 1,000,000, and 9.3 per cent over 1,000,000: maximum 4,952,000, minimum 7,500.

Bergey (1907) (Doane-Buckley test) gives the tests for eight cows: average 181,000, maximum 640,000, minimum 16,000. Two other cows were examined but the cells were massed to such an extent that the number of cells could not be counted.

Kendall (1907) (p. 177, smeared-sediment test) has given the tests of 168 normal cows which would have been exceedingly valuable if they had been made by a better method. He found that 138 out of the number gave tests under 400,000 and none over 2,000,000.

Stone and Sprague (1909) (pp. 240–42, Doane-Buckley test) give the record of 57 cows from the Vermont Agricultural Experiment Station herd as follows: average 403,000, maximum 8,800,000, minimum 13,000; samples from the morning milking. They also give the tests for two healthy cows (p. 243) followed throughout the entire period of lactation:

Cow No. 1, average of 546 tests, 112,203, maximum 2,110,000, minimum 10,000.

Cow No. 2, average of 621 tests, 84,341, maximum 1,444,000, minimum 6,000.

Hewlett, Villar, and Revis (1910) give several tests of individual cows using a modified form of the Savage test. Six cows (pp. 64–65) gave results ranging from 3,545,000 to 12,500, all but two of the tests being under 1,000,000. Six cows from another dairy (pp. 69–70) showed a maximum of 3,900,000 and a minimum of 9,500. All of the cows are reported to have been healthy at the time these examinations were made.

b. Another important set of results are those which deal with what may be termed market milk. These results represent samples where the milk of from several to many cows has been mixed and the milk has stood for several hours under a variety of conditions. Such samples would not be expected to show the extreme of variation that would be found in the milk of individual cows, but the sum total of the averages should be the same.

Doane (1905) (p. 209) gives the results of nine tests but does not state the source of the samples: average 950,000, maximum 3,200,000, minimum 292,000.

Savage (1906) (p. 131) examined 17 samples of mixed milk from a variety of sources: average 487,000, maximum 1,980,000, minimum 21,000.

Ward, Henderson, and Haring (1907) (p. 147) give the record of 60 tests of market milk: average 64,000, maximum 539,000, minimum 9,000.

Kendall (1907) (p. 177) gives the results of testing 87 samples of market milk in New York. Of these, 60 gave counts under 400,000 and eight were above 2,000,000.

Campbell (1909) (p. 11, Doane-Buckley test) gives the results of testing twenty samples of market milk: average 46,000, maximum 125,000, minimum 7,000.

Hewlett, Villar, and Revis (1910) report on a number of tests of herds of six or less cows. Weekly tests of the first selected herd of six for six months (p. 62) show results ranging from 11,000 to 1,528,000. Individual cows were taken out during the tests and newly calved cows substituted for them so that 13 individuals were used during the test. Similar tests of a second herd of six for nine months (p. 65) yielded tests from 17,000 to 1,735,000. This series was begun when the cows were well along in the period of lactation and were continued until all of the cows were dry. A third herd (p. 69) gave a maximum of 4,255,000 and a minimum of 136,000 during an eight-months test. A fourth herd of six, all newly calved (p. 73), gave a maximum of 4,360,000 and a minimum of 36,000 during a two-and-a-half-months test. The fifth herd of six (p. 78) during a period of nearly seven months showed a maximum of 1,258,000 and a minimum of 67,000. Herds 3 and 5 are reported to have been entirely healthy during the period of the tests but the other herds all included individuals which developed more or less abnormal conditions.

A comparison of these figures shows a surprising variation between the figures obtained by the different workers (cf. the figures given by Ward, Henderson, and Haring and those given by Stone and Sprague, for example). This can be explained in one of two ways. Either the cattle examined showed very different conditions or else the method used does not produce consistent results in the hands of different investigators. The probabilities are in favor of the second explanation, as is indicated by the discovery of the influence of preliminary heating on a sample by Russell and Hoffman (1908) and corroborated by the work of Campbell (1909), and of the use of formalin by Hewlett, Villar, and Revis (1910), as well as by the discovery of the uncertainty of the precipitation of the cells by the centrifuge by Prescott and Breed (1910).

The average given by Prescott and Breed (1910) for 46 samples of mixed milk obtained from as many different New England farms and determined by their new method is 1,485,000, maximum 10,690,000, minimum 60,000. The average of eight tests of market milk obtained from two of the highest class dealers of Boston was 2,850,000, maximum 5,210,000, minimum 1,310,000.

c. Certain other tests have been made to determine whether the different quarters of the udder vary as a unit or independently. Doane (1905) (p. 212) gives the results of testing three different cows in this way and comes to the conclusion that "the same variation that is found between individual cows is seen in the milk from different quarters of the same udder and there is considerable variation in the same quarter from day to day." An examination of two cows with a diseased quarter (p. 219) led him to conclude that one quarter may be seriously affected without influencing the other quarters.

Russell and Hoffman (1907*a*) (p. 241): "Seventy-two examinations made from the milk of individual quarters of the udder showed wide fluctuations in the milk of the same animal. It was noteworthy where any individual quarter showed a high cell content that this condition was observable through all portions of the milking."

Hewlett, Villar, and Revis (1910) give quite a body of evidence in their paper which agrees with the conclusions of Russell and Hoffman stated above. They note also a tendency to persistence of similar counts in the various quarters from day to day.

d. More investigators have tested to see whether there was any constant difference between the fore milk, middle milk, and strippings, sometimes using the mixed milk from all four quarters and sometimes testing each quarter separately.

Thus Doane (1905) (p. 212) tried to find milk without cells but found them in the fore milk, middle milk, and strippings of each quarter of the udder of a cow giving counts of but 1,000 to 5,000. He found the cells more numerous sometimes in the fore milk and sometimes in the strippings, with a considerable variation in conditions in different cases.

Savage (1906) (p. 137) found fewer cells in the middle milk of four cows than in the fore milk or strippings, but there was little difference between the latter two.

Russell and Hoffman (1907*a*) (p. 240) state that the results of testing 18 animals show that "strippings milk contains on an average three times as many cells as the fore or middle milk. As between fore and middle milk no regularity was observed. The increase in the strippings is, however, so marked that this factor should be taken into consideration in the matter of sampling."

Campbell (1909) (p. 14, modified Doane-Buckley test) made 14 tests of nine different cows in which a majority of tests show more cells in the strippings.

Hewlett, Villar, and Revis (1910) (pp. 60–61) report on six examinations but fail to find as striking results as Russell and Hoffman. Their conclusion is as follows: "From these results it is fairly evident that the cells appear practically uniformly throughout the milking, the slight increase in the strippings being probably caused by increased manipulation of the udder by the milker in stripping."

e. A more important question is whether there is any constant variation in number due to the period of lactation. There is a general belief that there is a decidedly larger number of cells present during the early days of lactation when the colostrum corpuscles are present than later, and that the increased number again makes its appearance when the animal is being dried off. These beliefs are founded on insufficient evidence, as will be seen from the following review:

Savage (1906) gives some data for 25 cows and concludes (p. 135) that there "appears to be no relation between the time interval and the number of leucocytes." One cow in the sixth day of lactation gave a count of 35,000.

Ward, Henderson, and Haring (1906) (pp. 152–54) give the following reports of examinations of fresh cows by the Doane-Buckley method:

Cow 1— 80,000, Udder indurated. Calved eight days ago.
Cow 2— 11,000, Udder indurated. Calved seven days ago.
Cow 3— 9,000, Fresh three days. Milk used.
Cow 4— 17,000, Fresh. Milk not used.
Cow 5—226,000, Fresh. Milk not used.
Cow 6—268,000, Fresh. Milk used.
Cow 7—453,000, Fresh. Milk used.
Cow 8— 17,000, Fresh.

Russell and Hoffman (1907*a*) (p. 241) make the following statements: "The effect of parturition was also studied, but examinations of colostrum milk could not be made on account of the presence of large numbers of colostral cells and of albumen. This condition persists for a period of three or four days, after which time microscopic examinations reveal no abnormal number of leucocytes." "No particular effect can be ascribed to the influence of lactation. A study of the history of the individual cows examined shows no regularity in the fluctuation as lactation advances. We have found wide variations in the number of cells from day to day and have frequently noted a high cell content in the earlier, as well as in the later, periods of lactation."

Pennington and Roberts (1908) (p. 77) make the statement that their evidence confirms the "assertion that pus makes its appearance if the cow is milked too long." In using the smeared-sediment method, they find that 75 per cent of the healthy animals show pus at the end of the milking period. However, no great weight can be given this statement because of the method used.

Stone and Sprague (1909) have made the most careful examination of two healthy cows, twice daily, throughout their whole period of lactation. Like Doane, they find the most surprising and unexpected variations from day to day and conclude that they must be due to transient and trivial causes. One cow varied from 6,000 to 1,444,000 and the other from 10,000 to 2,100,000 cells per cubic centimeter. They state that their highest counts in the case of one cow were obtained in the first two days of lactation and that four out of eight counts between 500,000 and 1,000,000 were obtained either during the first two days or the last two days of lactation. They say nothing about the time when they obtained the high counts in the case of the other cow, probably because the same relationship does not hold true.

Campbell (1909) (pp. 12–13, Doane-Buckley test) gives the record of two cows where samples were examined on alternate days for a period of two months, but little can be gathered other than the fact noted by Doane that there is great variation from day to day. One of the cows was an animal in her first period of lactation. She gave no very high counts in the twenty tests: average 16,500, maximum 81,000, minimum 2,000. The other cow was one suffering from an inflammation of the udder especially located in one quarter. The samples tested were the mixed milk from the remaining three quarters. The numbers are greater in this case than the other, but run over 500,000 in only two instances out of 20: average 180,600, maximum 720,000, minimum 14,000.

Hewlett, Villar, and Revis (1910) (pp. 62 and 73) made two series of tests to find whether the inclusion of newly calved cows in a herd increased the cell content of the mixed milk from the herd, but failed to find any marked influence. They give the following tests of fresh cows with the statement that they had been fresh from 7–14 days (p. 73): Cow 33 339,000; Cow 34 110,000 (contained trace of blood); Cow 35 36,000; Cow 36 415,000; Cow 37 4,360,000 (nine days since calving. Details given in regard to this case which developed a case of mastitis); Cow 38 99,000. These authors also give the results of examining a herd (p. 64) where some of the cows were being dried off, but failed to find any regular increase as the cows approached the end of their period of lactation. One cow far advanced in calf gave a very high and fluctuating count while three others which were barren showed low counts.

f. Stone and Sprague (1909) tried to determine whether there was any relation between the number of cells in the milk and the number of leukocytes in the blood but discovered none in the extended series of tests made.

g. It has been suggested several times that the age of the cow might influence the number of cells in the milk but little evidence has been submitted by anyone except Russell and Hoffman (1907*a*). On p. 253 they make the following statement: "From the studies made, it appears evident that the milks of older animals are more likely to show a high cell content than the younger stock and that frequently this condition is associated with a previous udder disturbance, which in some instances may leave a more or less marked indurated condition in the udder."

h. Russell and Hoffman (1907*a*) found no relation between the cell content and the breed of the animal.

i. One of the most important points of all, because of its bearing on sanitary problems, is the question whether there is any relation between high cell content and pathological conditions. It has been generally assumed, especially by health authorities, that such is the case. The foundation for this belief has been secured largely by the use of the Stokes smeared-sediment method with its various modifications. If now we rule this evidence out, as the authors of this paper believe they are justified in doing, because of the very great inaccuracy of these methods, this belief is found to rest on the following insufficient and contradictory evidence.

Doane (1905) (p. 213) states that "it has been demonstrated that when very great numbers of leucocytes are present in the milk there is evidently inflammation present in the udder." His examination of four cows (pp. 217–19) tends to support this statement except that in the case of Cow 86 it would appear that he has concluded that the cow had a diseased quarter because she showed a high cell count and he admits (p. 220) that "some radical and unexplained variations can be seen in the results of the count with the milk of Cow No. 86 from day to day."

Savage (1906) (p. 136) reports a cow suffering from infective mammitis whose milk showed an excessively high cell content.

Ward, Henderson, and Haring (1906) give an excellent list of tests of 47 cows where the udder was indurated or suffering from some pathological condition. These tests indicate very clearly that abnormal conditions do not necessarily produce large numbers of cells in the milk. The tests of 28 cows reported to be suffering from more or less indurated udders show four tests where the cells were so badly clumped as to be uncountable, three over 500,000, while 21 are under the 500,000 limit which has been so generally used as the standard. Their final conclusion (p. 156) is that "in view of all these observations we feel that the numerical determination of leucocytes in mixed milk, for the purpose of detecting the existence of mammitis among the cows of the dairy, does not rest on a firm experimental basis."

The work of Russell and Hoffman (1907*a*) on this point is the most careful and extended that we have. In comparing a long series of tests of 18 normal healthy cows with those from 11 cows showing more or less indurated conditions, they make the following statements (p. 247): "While it is undoubtedly true that cows suffering from udder trouble often do produce milk that is rich in leucocytes, it would seem equally true that high quantitative results were found with such frequency in milks from healthy animals as to vitiate the accuracy of a test based solely on a numerical foundation"; (p. 248): "In Group I (healthy cows) there are 11 cows whose counts never exceeded 500,000 and only three where the number was ever more than 1,000,000. Of the 11 animals showing an indurated condition, all but two gave counts above 1,000,000 with only one cow whose counts never exceeded 500,000." Some of these results were obtained with the unmodified Doane-Buckley test, in others the sample was subjected to preliminary heating. The detailed tests of three cows are given (p. 249), all of whom developed garget during the course of the tests. In the series given, it is noteworthy that many of the tests made while the animals were suffering from the attack were lower than those obtained before or after the attack, and the highest test (4,132,000) was obtained, not during the period of garget, but nearly three weeks before the attack and in the cow that had the lightest attack.

The cow which Campbell (1909) studied (Doane-Buckley test) for several weeks and which had one dry quarter, atrophied and very hard at the time the first samples were taken, shows but two counts over 500,000 and only two between 200,000 and 500,000 in a total of 20 counts, and yet he makes the statement that "the majority of them showed an extremely large number of leucocytes."

Hoffman (1909) has shown that the number of these cells may be increased by the injection of such inert materials as distilled water, physiological salt solution, or a boric-acid solution into the udder. The disturbances did not last over a very extended period of time.

Hewlett, Villar, and Revis (1910) investigated several cows during periods of garget. Their conclusion (p. 82) is as follows: "We are of the opinion that the cytological examination of milk does not admit of any inference of the existence of a diseased condition of the cows supplying the milk. It may point to the desirability of veterinary inspection, but gives no 'a priori' grounds for condemnation of the milk."

j. It has been suggested that certain physiological disturbances due to pregnancy, barrenness, or excitement due to heat have an influence on the number of these cells in the milk, but there is little satisfactory evidence that even indicates that such may be the case. It has also been claimed that the udder is so sensitive to disturbances of various kinds that a change of milkers, a change in the ration of the animal, undue excitement, feeding the animal during milking, bathing the udder in cold water, and the tuberculin test all produce a marked increase in the number of cells, but there is little or no evidence to show that such may be the case and it is very questionable whether all of these factors influence the cell content of the milk.

k. Another matter upon which considerable stress has been laid is that of the presence of fibrin in its relation to high cell counts. It is especially emphasized by Doane (1905), who feels after making an extended series of examinations that the presence of fibrin is a still better proof of diseased conditions than high cell content. He states: "The presence of fibrin combined with an abnormal number of leucocytes is the only satisfactory proof that inflammation exists in the udder."

Russell and Hoffman (1907*a*) (p. 252) fail to find such a close relationship between high cell content and fibrin. Nine out of 62 tests made where the cell count was less than 500,000 showed fibrin, while 12 out of 28 tests where the cell count was over 500,000 failed to show fibrin.

l. The relation between the number of these cells and the bacterial content of the milk both quantitative and qualitative has been discussed by various authors. The principal evidence from the quantitative standpoint has been furnished by Russell and Hoffman (1907*a*) and Hastings and Hoffman (1909). The former state (p. 250): "Out of 39 examinations there appeared to be no direct relation, although in some cases a high leucocyte count was accompanied by a high germ content. Quantitatively then, no relationship between the two factors above mentioned exists." "From the qualitative standpoint, however, there apparently was some connection, for it was noted that a high leucocyte count was usually associated with the presence of organisms presumably belonging to the streptococcus type." Hastings and Hoffman report on a long series of observations on two cows whose milk continuously showed a high germ content largely streptococci. Their milk also showed a high cell content, although both animals were apparently healthy.

The relationship between the presence of streptococci and a high cell content has been much discussed by various authors, only two of whom need to be considered here. Bergey (1907, and several earlier papers) thinks that there is a relationship between the two. Savage (1906) is equally convinced that there is none. The general opinion in this country seems to be that there is such a relationship, but no evidence has yet been produced either for or against this view which will stand a critical analysis.

ORIGINAL INVESTIGATIONS.

I. MATERIAL AND METHOD.

The present investigation has not dealt directly with all of the points mentioned in the review but does throw some light on most of them. The principal object has been to determine the normal conditions as revealed by the new method. Therefore normal, healthy cows have been selected for all of the work unless otherwise noted. The first part of the work was done largely by the junior author in the Biological Laboratory of Allegheny College and the samples used were obtained through the kindness of two milkmen in the neighborhood of Meadville, Pennsylvania. The herds from which the samples were obtained were kept under excellent conditions. During the time of the investigation the cattle were kept in the stable and were fed on ensilage corn, hay, and grain. The animals were Jerseys or grade Jersey and Durham. One of the herds had been tested with tuberculin and the reacting animals removed. The other herd was tested during the time of the investigation and six reacting animals were found out of the forty. Unfortunately it is impossible to state how many of these reacting animals are included in the reports.

The second part of the work was done by the senior author in Professor W. Fleishmann's laboratory in the Landwirtschaftliches Institut der Universität Göttingen. The senior author wishes to express his appreciation here of the kindly and sympathetic interest of Professor Fleishmann, who made it possible to carry out the investigation more completely than was at first thought possible. The principal samples used in this part of the work were either market milk samples from the Central Molkerei or from individual cows kept in Engelhardt's Kindermilchanstalt, in which the animals were kept under excellent hygienic conditions. The cattle here were largely of the Harz and Swiss types.

The method used in all cases for counting the cells was the direct method devised by Prescott and Breed (1910). This method has been found to be entirely practical and very simple in operation. The junior author found little difficulty with it and made nearly one-half of the tests reported. In all of the tests given here, duplicate smears were made and 100 fields of the oil immersion lens counted on each smear. The duplicate examinations do not show all of the agreement that could be desired, and it is possible that some means could be devised for measuring the small quantity of milk used (0.01 c.c.) more accurately. The use of larger quantities of milk makes it difficult to get even smears. The method was also found to be satisfactory for use in examining creams and skim milks as well as colostral milks, advantages not possessed by the earlier methods.

All samples were prepared for examination within a short time after milking, usually less than two hours.

2. TESTS OF COWS FROM NEAR MEADVILLE, PENNSYLVANIA.

Normal number of cells present in the milk of the average dairy cow.—Data from 37 cows, all of whom were apparently healthy and secreting milk of normal appearance. They were not subjected to a careful veterinary inspection at the time of the test and so the list possibly includes some animals where such an inspection would have revealed slight abnormalities. The list includes not more than six, and probably not more than three, animals that reacted to the tuberculin test.

Cow	Age Years	Period of Lactation Months	First Count	Second Count	Average No. of Cells per c.c.
1	3	8	800,000	640,000	720,000
2	2	5	425,000	370,000	400,000
3	6	8	410,000	410,000	410,000
4	8	8	660,000	500,000	580,000
5	12	9	510,000	480,000	495,000
6	6	10	2,800,000	2,640,000	2,720,000
7	7	10	1,030,000	1,050,000	1,040,000
8	4	5	590,000	410,000	500,000
9	3	4	470,000	440,000	455,000
10	9	7	50,000	50,000	50,000
11	2	1	540,000	710,000	625,000
12	7	9	420,000	580,000	500,000
13	4	3	1,250,000	1,050,000	1,150,000
14	2	4	1,050,000	810,000	930,000
15	2	3	790,000	820,000	805,000
16	3	8	760,000	670,000	715,000
17	6	9	960,000	860,000	910,000
18	6	9	480,000	500,000	490,000
19	7	8	5,630,000	1,730,000	3,080,000
20	6	8	1,130,000	1,070,000	1,100,000
21	8	9	670,000	500,000	585,000
22	2	5	720,000	580,000	650,000
23	7	8	5,810,000	4,870,000	5,340,000
24	6	9	1,510,000	1,700,000	1,600,000
25	2	8	1,510,000	1,100,000	1,305,000
26	6	9	1,620,000	1,060,000	1,340,000
27	8	9	400,000	300,000	350,000
28	8	8	630,000	320,000	475,000
29	7	9	450,000	290,000	370,000
30	8	7	580,000	480,000	530,000
31	..	..	1,160,000	1,395,000	1,125,000
32	..	..	220,000	240,000	230,000
33	..	..	1,280,000	1,150,000	1,215,000
34	..	..	3,695,000	3,560,000	3,630,000
35	..	..	1,785,000	1,680,000	1,730,000
36	..	..	3,850,000	3,640,000	3,745,000
37	..	..	1,135,000	1,265,000	1,200,000

This gives an average of 1,165,000 per cubic centimeter for the 37 cows, maximum 5,340,000, minimum 50,000; 30 per cent of the counts are below 500,000, 40 per cent between 500,000 and 1,000,000, and 30 per cent over 1,000,000. None of the milk was abnormal in appearance and it was used for delivery to customers.

No marked relation is shown by these tests between the number of cells and the age of the cow or between the number of cells and the period of lactation. The same remarkable variation occurs that has been noted by previous workers who have used the other methods. The fact that the average is much higher than any noted in the review of the previous work done was to be expected from the use of the new method. This average corresponds very well with the results obtained by Prescott and Breed (1910) for 46 samples of market milk where the average was 1,485,000 per cubic centimeter.

Number of cells present in the milk of the various quarters of the udder.—The samples were obtained by milking all of the milk of each quadrant separately and then sampling.

Cow	Quadrant	First Count	Second Count	Average No. of Cells per c.c.	Notes
38.......	R. F.	715,000	600,000	660,000	Fresh. First day of lactation. Average of all four quadrants —9,105,000 cells per c.c.
	R. H.	21,885,000	17,790,000	19,840,000	
	L. F.	14,225,000	14,600,000	14,410,000	
	L. H.	1,350,000	1,680,000	1,515,000	
39.......	R. F.	3,500,000	3,450,000	3,475,000	Fresh. Third day of lactation. Average of four quadrants—2,485,000 per c.c.
	R. H.	2,380,000	3,000,000	2,690,000	
	L. F.	2,780,000	3,510,000	3,145,000	
	L. H.	745,000	510,000	630,000	
40.......	R. F.	3,390,000	3,190,000	3,290,000	Within one week of the end of the lactation period. Average of four quadrants—3,865,000 per c.c.
	R. H.	3,145,000	2,770,000	2,960,000	
	L. F.	3,215,000	3,005,000	3,110,000	
	L. H.	5,920,000	6,285,000	6,100,000	
41.......	R. F.	220,000	210,000	215,000	Period of lactation—several months. Average of four quadrants—280,000 per c.c.
	R. H.	300,000	360,000	330,000	
	L. F.	525,000	375,000	450,000	
	L. H.	115,000	150,000	135,000	
42.......	R. F.	70,000	55,000	65,000	Period of lactation—several months. Average of four quadrants—60,000 per c.c.
	R. H.	35,000	55,000	45,000	
	L. F.	50,000	50,000	50,000	
	L. H.	65,000	80,000	70,000	
43.......	R. F.	675,000	575,000	625,000	Period of lactation—several months. Average of four quadrants—305,000 per c.c.
	R. H.	140,000	155,000	150,000	
	L. F.	400,000	365,000	380,000	
	L. H.	65,000	55,000	60,000	
44.......	R. F.	700,000	655,000	675,000	Period of lactation—several months. Average of four quadrants—905,000 per c.c.
	R. H.	660,000	815,000	740,000	
	L. F.	625,000	680,000	650,000	
	L. H.	1,710,000	1,410,000	1,560,000	
45.......	R. F.	245,000	160,000	200,000	Fresh. R. H. quarter injured so that it gave no milk. R. F. quarter affected and milk bloody. Cow 7 yrs. old.

This table brings out some interesting conditions. There is the widest variation between the four quadrants, indicating that each quadrant acts independently. No one quadrant gives a constantly higher or lower count than the others. The tests of the cows in the early and late stages of lactation are especially important and will be referred to later.

Number of cells present in the fore milk, middle milk, and strippings of the average dairy cow.—Apparently normal and healthy except as noted.

Cow		First Count	Second Count	Average No. of Cells per c.c.	Notes
46.......	Fore milk	490,000	500,000	495,000	Mixed milk of all four quadrants.
	Middle milk	445,000	425,000	435,000	
	Strippings	850,000	745,000	800,000	
47.......	Fore milk	12,420,000	12,635,000	12,525,000	Right hind quarter of a tubercular cow.
	Middle milk	12,295,000	12,875,000	12,585,000	
	Strippings	10,490,000	12,745,000	11,620,000	
47.......	Fore milk	2,385,000	2,150,000	2,270,000	Left hind quarter of the same cow.
	Middle milk	2,600,000	2,485,000	2,540,000	
	Strippings	17,995,000	17,845,000	17,920,000	
48.......	Fore milk	340,000	345,000	345,000	Mixed milk from all four quadrants.
	Middle milk	710,000	505,000	605,000	
	Strippings	2,800,0000	2,790,000	2,795,000	
49.......	Fore milk	220,000	175,000	195,000	Mixed milk of all four quadrants.
	Middle milk	120,000	150,000	135,000	
	Strippings	795,000	940,000	870,000	
50.......	Fore milk	590,000	655,000	620,000	Mixed milk of four quadrants. Cow 6 yrs. old, in good condition. Fresh 5 days.
	Middle milk	300,000	210,000	255,000	
	Strippings	995,000	955,000	975,000	
51.......	Fore milk	125,000	155,000	140,000	Mixed milk of all four quadrants. Cow 4 yrs. old. Fresh two days.
	Middle milk	Sample brok'n	....	....	
	Strippings	1,150,000	715,000	930,000	
52.......	Fore milk	....	....	....	Mixed milk four quadrants. Cow 4 yrs. old, good condition. Within two weeks of end of lactation period.
	Middle milk	155,000	145,000	150,000	
	Strippings	200,000	185,000	195,000	

All of these tests, with a single exception, show decidedly more numerous cells in the strippings than in the fore or middle milk. The single exception noted (No. 47, right hind quarter) shows but a few less cells in the strippings and there is reason for believing that this may be due to experimental error (note duplicate tests) rather than to the real condition. The fact that some counts show much greater relative differences than others is probably at least partially explained by differences in the thoroughness of milking. Differences between the fore milk and the middle milk are possibly due to differences in the thoroughness of the previous milking.

These tests give some further data regarding early and late stages of lactation. There is no reason for believing that the large counts obtained from the tubercular cow were due to that rather than to other causes.

3. TESTS OF COWS FROM GÖTTINGEN, GERMANY.

Number of cells found in the milk of two healthy cows during a period of four weeks.—Samples of milk from the mixed milk of the entire morning milking were received from the Kindermilchanstalt daily. The two cows were selected as the most normal animals possible to obtain and without any reference to the cell content of their milk. Both had been under competent veterinary inspection and showed no known history

Cow 54				Cow 55			
Date	First Count	Second Count	Average No. of Cells per c.c.	Date	First Count	Second Count	Average No. of Cells per c.c.
Nov. 9......	510,000	470,000	490,000	Nov. 9......	2,385,000	2,440,000	2,415,000
10......	260,000	300,000	280,000	10......	1,900,000	1,860,000	1,880,000
11......	455,000	535,000	495,000	11......	1,610,000	1,880,000	1,745,000
12......	335,000	355,000	345,000	12......	1,040,000	1,020,000	1,030,000
13......	....	....	....	13......	....	....	....
14......	260,000	180,000	220,000	14......	3,350,000	3,580,000	3,465,000
15......	435,000	465,000	450,000	15......	2,500,000	2,930,000	2,715,000
16......	300,000	270,000	285,000	16......	1,240,000	1,250,000	1,245,000
17......	290,000	225,000	255,000	17......	1,985,000	1,825,000	1,905,000
18......	620,000	450,000	535,000	18......	1,800,000	1,470,000	1,635,000
19......	280,000	230,000	255,000	19......	1,765,000	1,480,000	1,625,000
20......	425,000	415,000	420,000	20......	2,280,000	2,460,000	2,370,000
21......	1,185,000	925,000	1,055,000	21......	1,785,000	2,015,000	1,900,000
22......	1,000,000	810,000	905,000	22......	1,480,000	1,220,000	1,350,000
23......	460,000	390,000	425,000	23......	1,615,000	1,905,000	1,760,000
24......	205,000	225,000	215,000	24......	1,780,000	1,570,000	1,675,000
25......	505,000	445,000	475,000	25......	1,165,000	1,305,000	1,235,000
26......	320,000	350,000	335,000	26......	780,000	1,110,000	945,000
27......	455,000	445,000	450,000	27......	3,120,000	2,960,000	3,040,000
28......	270,000	240,000	255,000	28......	2,090,000	2,120,000	2,105,000
29......	810,000	700,000	755,000	29......	1,005,000	925,000	965,000
30......	700,000	660,000	680,000	30......	5,920,000	6,030,000	5,975,000
Dec. 1......	360,000	410,000	385,000	Dec. 1......	2,200,000	2,030,000	2,110,000
2......	740,000	920,000	830,000	2......	990,000	780,000	885,000
3......	940,000	1,080,000	1,010,000	3......	3,930,000	3,500,000	3,715,000
4......	615,000	685,000	650,000	4......	1,940,000	1,820,000	1,880,000
5......	460,000	390,000	425,000	5......	1,060,000	1,100,000	1,080,000
6......	810,000	1,150,000	980,000	6......	2,400,000	2,310,000	2,355,000
7......	930,000	960,000	945,000	7......	2,990,000	3,120,000	3,055,000
8......	710,000	710,000	710,000	8......	1,860,000	1,980,000	1,920,000

	Butter Fat	Total Solids	Specific Gravity		Butter Fat	Total Solids	Specific Gravity
Nov. 22......	3.4%	12.068%	1.0309	Nov. 21......	3.4%	11.767%	1.0297
Dec. 8......	3.6%	12.283%	1.0308	Nov. 22......	3.95%	12.653%	1.0306
				Nov. 28......	3.4%	11.867%	1.0301
Cow No. 54—Average of 29 tests = 535,000				Cow No. 55—Average of 29 tests = 2,070,000			

of garget or other troubles. Cow 54 was a cross between a Harz and Glaner strain, 7 years old, had calved March 6, 1910, and was giving 10½ liters per day on November 1. She had been tested with tuberculin on March 20. Better than the average cow of the herd, but not one of the best cows. Cow 55 was a Harz cow, 7 years old, had calved June 1, 1910, was tested with tuberculin June 14, and was giving 8 liters per day on November 1. She had had twin calves, both normal, and was not giving as much milk as she had in previous lactation periods. The udder was said to be smaller and more fleshy than formerly. A careful veterinary inspection of these cows on December 9 failed to disclose the slightest abnormal or pathological conditions. The fodder was hay, clover, and dry grain.

From November 30 to December 8, inclusive, the milk of Cow 55 was examined in greater detail to discover the conditions prevailing in the four quadrants of the udder. Unfortunately, just at this time there was a change of milkers and the instructions were not carried out perfectly, in that a varying amount of strippings was not included in the samples. This undoubtedly caused an equally variable error in all of these tests, the numbers being smaller than they otherwise would have been. This is also the explanation of the low fat content of the milk. By chance, the first test from the four quadrants was made on the morning when the mixed milk contained the greatest number of cells found during the 29 tests.

Date	Quadrant	First Count	Second Count	Average No. of Cells per c.c.	Butter Fat	Total Solids	Specific Gravity
Nov. 30. .	R. H.	21,750,000	23,250,000	22,500,000	2.7%	10.650%	1.0286
	L. H.	1,165,000	1,315,000	1,240,000	2.6%	11.283%	1.0316
	R. F.	2,665,000	2,455,000	2,560,000	2.6%	10.932%	1.0302
	L. F.	985,000	915,000	950,000	2.7%	11.178%	1.0307

Average of four quadrants—6,810,000. Test from mixed milk—5,975,000.

Date	Quadrant	First Count	Second Count	Average No. of Cells per c.c.	Butter Fat	Total Solids	Specific Gravity
Dec. 1. . .	R. H.	14,250,000	14,870,000	14,560,000	The milker forgot to make samples from the regular morning milking and drew these at 10 A.M.		
	L. H.	2,610,000	2,640,000	2,625,000			
	R. F.	3,820,000	4,080,000	3,950,000			
	L. F.	1,990,000	1,980,000	1,985,000			

Average—5,780,000. Mixed milk—2,115,000. This latter was from the regular morning milking and contained all of the strippings.

Date	Quadrant	First Count	Second Count	Average No. of Cells per c.c.	Butter Fat	Total Solids	Specific Gravity
Dec. 2. . .	R. H.	1,600,000	1,520,000	1,560,000	2.0%	10.463%	1.0312
	L. H.	710,000	630,000	670,000	3.1%	11.733%	1.0310
	R. F.	800,000	770,000	785,000	2.6%	11.183%	1.0312
	L. F.	330,000	350,000	340,000	2.3%	11.024%	1.0320
Average—840,000. Mixed milk—885,000.					2.55%	11.123%	1.0312

Date	Quadrant	First Count	Second Count	Average No. of Cells per c.c.	Butter Fat	Total Solids	Specific Gravity
Dec. 3. . .	R. H.	3,265,000	3,435,000	3,350,000	2.2%	10.653%	1.0310
	L. H.	2,430,000	2,560,000	2,495,000	2.55%	11.073%	1.0310
	R. F.	5,125,000	5,185,000	5,155,000	2.35%	10.733%	1.0306
	L. F.	2,860,000	2,880,000	2,870,000	2.1%	10.834%	1.0322
Average—3,465,000. Mixed milk—3,715,000.					2.3%	10.873%	1.0314

Dec. 4...	R. H. L. H. R. F. L. F.	3,750,000 1,235,000 2,540,000 1,430,000	3,500,000 1,225,000 3,130,000 1,260,000	3,625,000 1,230,000 2,835,000 1,345,000	2.3% 2.4% 2.5% 2.3%	10.999% 11.394% 11.113% 10.973%	1.0319 1.0330 1.0314 1.0318	
Average—2,260,000. Mixed milk—1,880,000.					2.3%	11.149%	1.0325	
Dec. 5...	R. H. L. H. R. F. L. F.	2,160,000 1,570,000 1,010,000 750,000	2,040,000 1,540,000 960,000 730,000	2,100,000 1,555,000 985,000 740,000	1.1% 2.1% 2.0% 1.6%	9.584% 10.909% 10.413% 10.409%	1.0320 1.0325 1.0310 1.0329	
Average—1,345,000. Mixed milk—1,080,000.					1.8%	10.574%	1.0326	
Dec. 6...	R. H. L. H. R. F. L. F.	6,390,000 1,110,000 650,000 1,650,000	6,420,000 1,100,000 740,000 1,570,000	6,405,000 1,105,000 695,000 1,610,000	2.0% 2.15% 2.4% 2.0%	10.112% 10.969% 11.043% 10.798%	1.0298 1.0325 1.0316 1.0325	
Average—2,455,000. Mixed milk—2,355,000.					2.1%	10.658%	1.0315	
Dec. 7...	R. H. L. H. R. F. L. F.	2,310,000 2,350,000 3,930,000 2,380,000	2,540,000 2,530,000 4,040,000 2,150,000	2,425,000 2,440,000 3,985,000 2,265,000	2.4% 2.6% 2.25% 1.8%	10.717% 11.409% 10.663% 10.549%	1.0303 1.0321 1.0308 15.302	1 liter 1½ liter 1 liter 1½ liter
Average—2,780,000. Mixed milk—3,055,000.					2.3%	10.873%	1.0314	

Two days later, after the explanation of the low fat content had been discovered, a more thorough test of the morning milking was made. The milk from each quadrant was milked as usual and kept separate. The same was done for the strippings that accumulated after the first milking. The tests follow:

Date	Quadrant	First Count	Second Count	Average No. of Cells per c.c.	Butter Fat	Total Solids	Specific Gravity	
Dec. 9...	R. H. L. H. R. F. L. F.	2,390,000 2,380,000 1,540,000 1,500,000	2,380,000 2,510,000 1,485,000 1,475,000	2,385,000 2,445,000 1,515,000 1,490,000	2.9% 2.9% 2.7% 2.8%	11.418% 11.693% 11.529% 11.599%	1.0307 1.0318 1.0321 1.0319	1165 c.c. 1175 c.c. 1020 c.c. 960 c.c.

The tests for the strippings follow:

	R. H. L. H. R. F. L. F.	4,760,000 5,255,000 3,470,000 2,685,000	4,800,000 5,395,000 3,285,000 2,855,000	4,780,000 5,325,000 3,375,000 2,770,000				104 c.c. 100 c.c. 96 c.c. 110 c.c.

Test of entire milk, strippings included.

		2,385,000	2,495,000	2,440,000	3.15%	11.743%	1.0308	4731 c.c.

This last test shows what a difference there is in the cell content of the middle milk and strippings. The cell count of the entire mixed milk is very markedly increased by the inclusion of the strippings. The principal conclusions to be drawn from these tests will be referred to later. Attention is called to the test of 22,500,000 cells per cubic centimeter in the right hind quarter of Cow 55 on November 30 as well as to many other tests which would be regarded as extremely high by the men who have used the older methods of counting the cells. In this connection, it should also be remembered that the tests from November 30 to December 9 for Cow 55 are lower than they would have been if the strippings had been included. Yet there is no sufficient reason disclosed for regarding this milk as in the slightest degree abnormal. Certainly the milker and the dealer both honestly believed it to be of the highest quality and it was handled in the cleanliest manner possible and sold for feeding babies.

Number of cells in each quadrant of the udder of a single healthy cow during a period of five weeks.—Samples from the entire milk obtained from the right hind quarter were taken from the evening milking daily and from the remaining three quadrants twice weekly. This cow (No. 56) was selected from the same herd as 54 and 55 and in the same way as were these animals. She was a Glaner cow, four years old, had calved June 1, 1910, was tuberculin tested on June 14, and was giving 13¾ liters per day on November 1. One of the best cows in the herd. In order to condense the report the duplicate counts will not be given in this case.

Cow 56.

Date	R. H.	L. H.	R. F.	L. F.	Notes
Nov. 3	2,500	5,000	230,000	2,500	The milk from three quadrants proved to have the fewest cells present that have been found by this new method. Where the figure given is 5,000, it means that only one cell was seen in 100 fields of the oil immersion lens. 2,500 means that no cells were seen in 100 fields but an examination with a lower power showed that cells were present.
4	....	....	....	....	
5	2,500	....	....	....	
6	2,500	....	....	....	
7	15,000	....	....	....	
8	15,000	20,000	530,000	10,000	
9	7,000	....	....	....	
10	20,000	....	....	....	
11	25,000	15,000	580,000	10,000	
12	2,500	....	....	....	
13	25,000	....	....	....	
14	25,000	....	....	....	
15	40,000	15,000	1,375,000	70,000	Butter fat 4.4 per cent, total solids 14.068 per cent, spec. grav. 1.0341 in R. H. (Nov. 15).
16	25,000	....	....	....	
17	7,500	....	....	....	
18	12,500	35,000	605,000	20,000	
19	15,000	15,000	780,000	20,000	
20	80,000	20,000	390,000	10,000	Morning milking. All others from evening milking.
	20,000	....	....	....	
21	15,000	....	....	....	
22	35,000	40,000	1,045,000	25,000	

Cow 56.—*Continued.*

Date	R. H.	L. H.	R. F.	L. F.	Notes
Nov. 23	30,000	....	....	....	Butter fat 4.6 per cent, total solids 14.059 per cent, spec. grav. 1.0331 in R. H. (Nov. 23).
24	200,000	....	....	....	
25	65,000	30,000	650,000	5,000	
26	10,000	....	....	....	
27	30,000	....	....	....	
28	12,500	....	....	....	
29	20,000	5,000	755,000	40,000	
30	125,000	....	....	....	
Dec. 1	25,000	....	....	....	
2	10,000	25,000	1,110,000	20,000	
3	10,000	....	....	....	
4	7,500	....	....	....	
5	20,000	....	....	....	
6	15,000	5,000	270,000	5,000	
7	20,000	....	....	....	Butter fat 3.7 per cent, total solids 13.427 per cent, spec. grav. 1.0349 in R. H. (Dec. 8).
8	10,000	....	....	....	

The average for the twelve days on which all four quadrants were tested is 190,000 cells per c.c.

The two charts show these results graphically and in a way that shows their real character much better than the figures. The last part of the curve for Cow 55 has been drawn in dotted lines to call attention to the fact that the results on which it is based are not as satisfactory as the others. The random selection of three cows proved very happy, for Cows 55 and 56 stand at opposite extremes so far as numbers of cells are concerned, while Cow 54 stands in an intermediate position.

Through the courtesy of Dr. George Wiegner, of the Landwirtschaftliches Institut der Universität Göttingen, samples were obtained from Cows 54, 55, and 56 on January 13 and 16, 1911, and forwarded to the senior author of this paper.

Cow 54: This cow met with an accident a few days before the samples were secured. A neighboring cow stepped upon her udder, bruising the left forward quadrant so that it gave bloody milk. The tests of the milk drawn from this quadrant on the morning of January 16 are as follows: 17,000,000–21,000,000; average, 19,000,000 cells per cubic centimeter. Only 50 fields of the microscope were counted in this case. There were large numbers of red blood corpuscles present in the smears and a great number of cell fragments, so that there is a very large error in the count.

The mixed milk of the remaining three quarters tested as follows: 760,000–660,000, average 710,000 cells per cubic centimeter. This number is very much the same as had been found previously in the milk of this cow. The number does not seem to have been influenced greatly by the bruising of the one quadrant.

Cow 55: Whole milk from morning milking of January 13, 3,160,000–2,840,000; average 3,000,000 cells per cubic centimeter. January 16, 3,000,000–3,020,000; average 3,010,000 cells per cubic centimeter. These two tests are both much higher than the average for this cow, and yet a glance at Chart 1 will show that similar tests might have been obtained several times during the previous month if taken at similar three-day intervals.

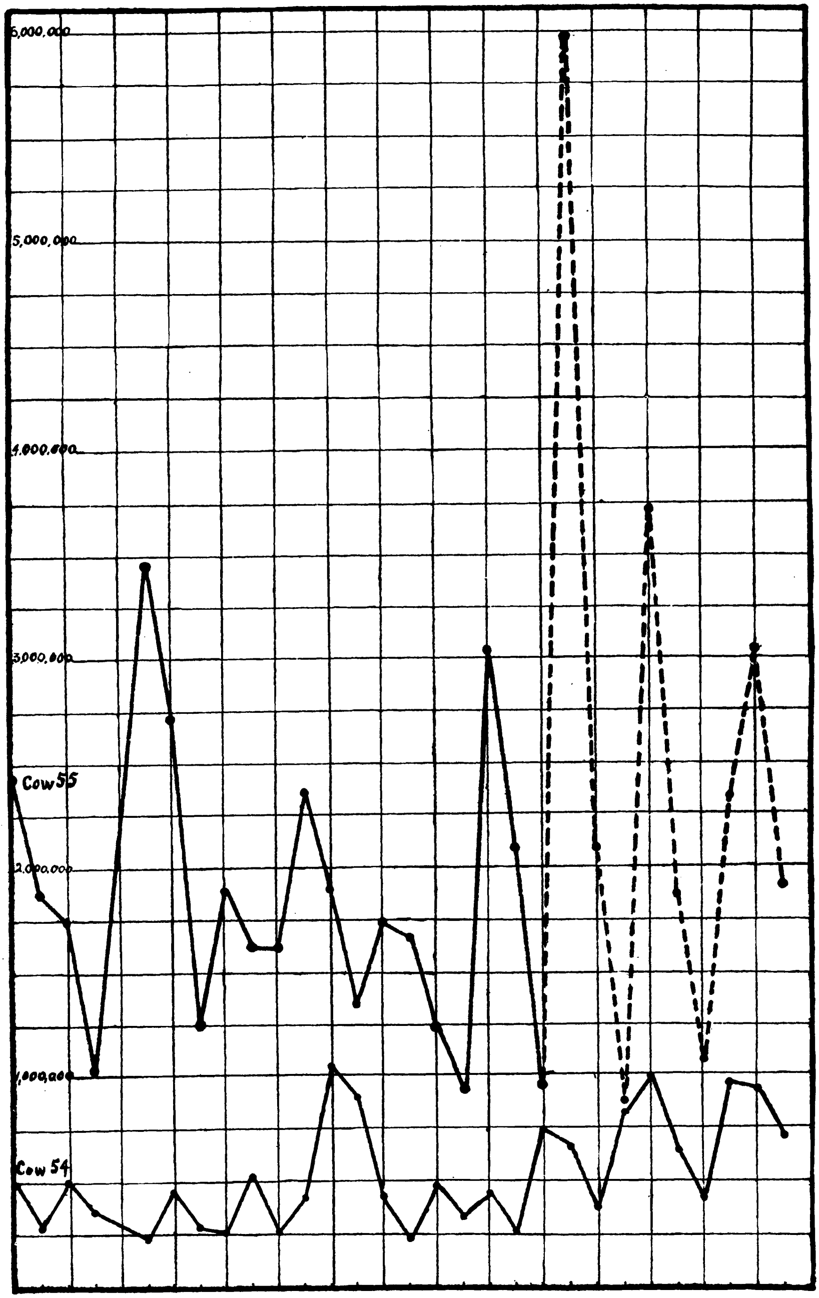

CHART 1.—Daily variation in the cellular content of the milk of Cows Nos. 54 and 55 from November 9 to December 8, 1910. Each vertical space represents 250,000 cells per cubic centimeter. Each horizontal space represents a two-day interval.

Cow 56: Separate quadrants, evening milking.

	January 13			January 16		
			Average			Average
R. H.......	40,000	40,000	40,000	90,000	65,000	75,000
R. F.......	50,000	40,000	45,000	30,000	40,000	35,000
L. H.......	3,160,000	2,810,000	2,985,000	865,000	1,025,000	945,000
L. F.......	30,000	15,000	22,000	30,000	40,000	35,000

It will be noted that all of the counts are somewhat higher than during the previous month.

Important points to notice are: first, that the cow giving the greatest quantity of milk discharged the least number of cells both actually and relatively. So striking is this difference that if the whole number of cells discharged by the right hind quarter of Cow 56 in the 36 milkings tested had been discharged at a *single milking* and that in a liter of milk instead of in the $1\frac{3}{4}$ liters

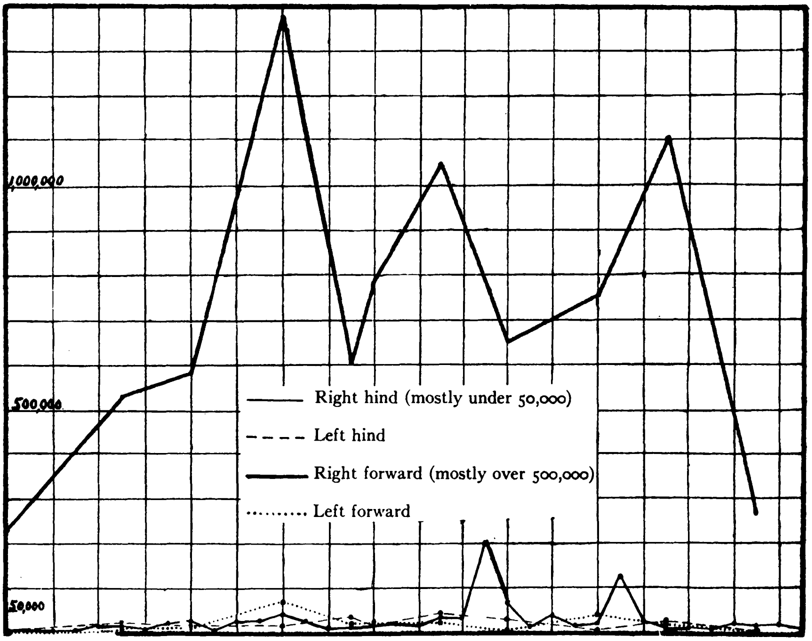

CHART 2.—Variation in the cellular content of the milk from the four quadrants of the udder of Cow No. 56 from November 3 to December 8, 1910. Each vertical space represents 100,000 cells per cubic centimeter. Each horizontal space represents a two-day interval.

actually secreted, the number of cells would have been approximately 2,000,000 per cubic centimeter, that is, a number which was the average number of cells found in the milk of Cow 55. In the extreme case, one quadrant of Cow 55 liberated almost as many cells at a *single milking* as the hind quarter of Cow 56 would have done in the *half of an entire lactation period* if the same low rate found in the period tested prevailed. Such facts as these are of importance in interpreting histological and physiological phenomena. The histological descriptions of the udder have been written by men who have necessarily studied but a small number of udders. No one of them has had any idea as to the number of cells which the udders studied were secreting at the time they were obtained for study. Is it any wonder that one histologist reports that cell divisions are common and another that they are rare or absent?

Second, there seems to be something of a cyclic variation in the curves of Cows 54 and 55. The time interval between maxima or between minima is almost always three days, with some two-day and some four-day intervals. The curve is ascending 20 days, descending 36 days, a ratio of almost one to two. In other words, there seems to be a tendency to jump suddenly to a maximum, usually in one day, while the descent to a minimum takes two or three days. Whether these points are of real significance or not remains to be seen.

Third, in spite of the enormous and sudden variations there seems to be a certain constancy for all three of the cows. This was sufficiently marked so that it was always possible to identify the three daily samples without recourse to the labels. This constancy held also for the separate quadrants of the udder in Cow 56.

Fourth, the greatest range of variation occurred in the cow which had the highest average number of cells in her milk, and the least range in the cow with the lowest average.

Number of cells in the market milk of Göttingen.—During the course of these investigations, market milk samples were tested seven times. All samples came from the Central Molkerei, but on different days. This milk had been purified by passing through a low speed separator which must have removed some of the cells, especially as the milk was warmed to 40° C. before passing through the separator. It should be noted here that the samples of market milk reported on by Prescott and Breed (1910) (p. 640) had been subjected to similar treatment except that they had not been subjected to the previous warming before passing through the separator. The tests of the Göttingen milk follow:

1,540,000
4,185,000
1,885,000
1,645,000
2,205,000
1,980,000
1,980,000
Average = 2,200,000

The comparison of the average with those obtained by Prescott and Breed shows that the conditions in Boston and Göttingen are not markedly different.

Goats' milk.—Tests of the milk of two goats showed the following results:

Goat 1, Age 3 yrs., Lambed April 1. Tested November 1, 425,000 cells per c.c.
Goat 2, Age 5 yrs., Lambed April 1, Tested November 1, 2,245,000 cells per c.c.

Both reported to be normal and healthy.

Fresh cows.—The scattered evidence presented in these pages concerning colostral milk may be presented in brief form here.

Cow 38—	9,105,000.	First day. Number given is the average of the tests of the individual quadrants as follows: R. F.—660,000, R. H.—19,840,000, L. F.—14,410,000, L. H.—1,515,000.
Cow 39—	2,485,000.	Third day. Ditto: R. F.—3,475,000, R. H.—2,690,000, L. F.—3,145,000, L. H.—630,000.
Cow 45—	200,000.	In R. F. quadrant. Fresh. This cow had been injured in the R. H. quadrant so that this quarter gave no milk and the quarter tested gave milk that was somewhat bloody. Cow 7 yrs. old.
Cow 50—	620,000.	Fore milk. Cow fresh five days. 6 yrs. old.
	255,000.	Middle milk.
	975,000.	Strippings.
Cow 51—	140,000.	Fore milk. Fresh two days. 4 yrs. old.
	930,000.	Strippings.
Cow 57—	1,535,000.	Second milking after calving. Full blood Holstein, 3 yrs. old. Udder indurated but not seriously. Reported by Breed (1910).

It will be seen that not one of these tests shows numbers of cells in excess of numbers reported elsewhere for apparently normal cows in later stages of lactation. Even Cow 38 does not reach the numbers given for Cow 55.

End of lactation period.—Only two tests bear directly on this point.

Cow 40—	3,865,000.	Within one week of the end of the lactation period. The number given is the average of tests of the individual quadrants as follows: R. F., 3,290,000, R. H., 2,960,000, L. F., 3,110,000, L. H., 6,100,000.
Cow 52—	150,000.	Middle milk. Within two weeks of the end of the lactation.
	195,000.	Strippings. Cow 4 yrs. old.

Neither of these tests is exceptionally different from apparently normal conditions.

Pathological conditions.—No effort was made to examine such material. Cow 47 and the two fresh cows (45 and 57) reported just above are the only animals which were examined which showed pathological conditions. The tests of these animals do not indicate much, but they do show that further investigation must be made before the commonly accepted conclusions on this point can be regarded as proved.

CONCLUSIONS.

In view of the fact that these investigations seem to show that nearly all of our beliefs regarding the presence of cellular elements in milk will need to be revised, a conservative statement of our knowledge concerning their number and significance has been deemed of value.

1. The number of cellular elements in cows' milk varies from numbers so few as to be almost negligible (less than 5,000 per cubic centimeter) to 20,000,000 and more per cubic centimeter in milk which we have good reason for calling normal. According to the investigations thus far available, nearly one-third of normal healthy cows show a cell content in their milk less than 500,000 per cubic centimeter, somewhat more than one-third show a cell content between 500,000 and 1,000,000 per cubic centimeter, while nearly one-third give milk containing more than 1,000,000 cells per cubic centimeter. There is no evident reason for regarding the discharge of these cells in the numbers stated as indicating pathological conditions. In certain other skin glands, notably the sebaceous glands, the secretion is very largely or entirely cellular and it is not surprising to find similar conditions in glands which are possibly homologous structures.

2. Competent evidence to support the common statements that colostral milk and milk from animals nearly dry contain larger numbers of cells than milk obtained during other periods of lactation is lacking. Striking variations in the number of cells occur from day to day, and there is some evidence to show that this variation may be cyclic in nature. Individual cows maintain a certain constancy of numbers for several weeks at a time, at least. It is possible that the number of cells may be correlated to some degree with the amount of milk secreted, udders which give large amounts discharging fewer cells than where less milk is secreted.

3. The range of variation between individual quarters of a single udder is apparently as great as the range of variation between different cows. Whether or not they vary absolutely independently of each other is not clear, but the relationship does not seem to be close.

4. There is good reason for believing that there is always an increase of the number of cells in the strippings where the milking is done thoroughly. No constant relation between the fore milk and the middle milk is apparent.

5. The cause of the normal variations is not clear from the evidence at hand and probably will not be found until the nature of the cells is clear. Conclusive evidence to show whether such suggested causes as age, period of lactation, race of cow, barrenness, pregnancy, heat, the ration used, undue excitement, etc., have or have not an influence on the number of these cells is not at hand.

6. The numerical relation between these cells and pathological conditions is not at all clear. The statement that large numbers of these cells in the milk indicate a pathological condition is as unjustified by the evidence as is the converse that all pathological conditions produce an increased number of cells. Such evidence as there is indicates that very evident pathological conditions do not necessarily produce milk which has even an average number of cells for normal milk. Yet it is undoubtedly true that some pathological conditions do produce excessively large numbers of cells in the milk.

7. The evidence that there is a relation between the bacterial content of the udder, especially streptococcic, and the number of these cells is very inconclusive when subjected to a critical analysis. It may be that such a relationship holds true, but a fair verdict must be the old Scotch one of "not proven."

8. Inasmuch as it is evident from these investigations that where market milk is the mixed milk of any large number of cows the number of cells is bound to be considerably in excess of 1,000,000 per cubic centimeter, it is evident that all of the present numerical standards must be discarded. As there is at present no good evidence to show that the number of these cells has more

than minor hygienic significance, if any at all, there is no reason for suggesting other standards.

It may be said that these conclusions do not give sufficient weight to the previous investigations on this subject. Yet it seems to the authors of this paper that greater weight cannot be given in view of the evidence presented here and elsewhere.

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NOTE.

ERRATA IN ARTICLE BY PRESCOTT AND BREED, *Jour. Infect. Dis.*, 1910, 7, pp. 632 ff.:

P. 634, l. 14: "0.005" should read "0.0002 or 1/5000."

P. 638, l. 6: "eight half-pint" should read "cans containing 16 ½ pints."